UNIVERSITI PUTRA MALAYSIA

SOME ASPECTS OF THE BIOLOGY AND CONTROL OF VASCULAR STREAK DIEBACK PATHOGEN (ONCOBASIDIUM THEOBROMAE) OF COCOA

LAM CHIN HEE

It is hereby certified that we have read this thesis entitled 'Some Aspects of the Biology and Control of Vascular Streak Dieback Pathogen (*Oncobasidium theobromae*) of Cocoa' by Lam Chin Hee, and in our opinion it is satisfactory in terms of the scope, quality, and presentation as partial fulfilment of the requirements for the degree of Master of Agriculture Science

.....
SULAIMAN M. YASSIN, Ph.D.
Professor/Dean of Graduate Studies
Universiti Pertanian Malaysia
(Chairman Board of Examiners)

.....
B.E.J. WHEELER, Ph.D., D.Sc.
Department of Pure & Applied Biology
Imperial College Ascot
England
(External Examiner)

.....
ABDUL RAHMAN RAZAK, Ph.D.
Associate Professor/Deputy Dean
Faculty of Agriculture
Universiti Pertanian Malaysia
(Internal Examiner)

.....
ZAINAL ABIDIN MIOR AHMAD, M.Agr.Sc.
Faculty of Agriculture
Universiti Pertanian Malaysia
(Internal Examiner/Supervisor)

This thesis was submitted to the Senate of Universiti Pertanian Malaysia and was accepted as partial fulfilment of the requirements for the degree of Master of Agricultural Science.

Date: 21 JUL 1988

(SULAIMAN M. YASSIN, Ph. D.)
Professor/Dean of Graduate Studies

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A thesis submitted in partial fulfilment of the requirements
for the degree of Master of Agricultural Science
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calli was best expressed using a 2mm mycelial disc obtained from primary isolates of O. theobromae as inoculum, with cultures incubated at temperature of 25°C under complete darkness or 10/14 hours of light/darkness.

Changes in histology, activity of peroxidase and phenolic contents were determined in cocoa calli derived from susceptible (Amelonado) and resistant (KKM 25 and Sca 6 x I 466) genotypes inoculated with O. theobromae compared to uninoculated calli. Occurrence of thick-walled cells, general increase in peroxidase activity, decrease in content of phenolic compounds (o-dihydric phenols, leucoanthocyanins and flavanols) and lower flavanols/total phenols ratio were associated with inoculated calli derived from resistant genotypes. In the inoculated calli of the susceptible genotype, no histological change was observed. However, biochemical changes recorded were the reverse of the resistant genotypes.

In vitro screening of systemic fungicides belonging to the triazole group by the poisoned-agar method showed high fungitoxic activity against O. theobromae. The ED_{50} values (in ascending order) of the fungicides, DPX-H6573, propiconazole, LS840608, triadimefon, triadimenol and RH-3866 were 0.33, 0.36, 0.61, 0.63, 0.70, 1.12 ppm a.i.

The use of a dual culture technique of cocoa callus with O. theobromae for studying fungitoxicity of a systemic fungicide, propiconazole, was evaluated. Progressive inhibition of the fungus was observed with increasing dosage levels of the test fungicide.

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Pensporulaan kulat di atas medium agar belum pernah dilaporkan. Satu kaedah telah diperkembangkan untuk pensporulaan dan penghasilan basidiospora O. theobromae yang banyak di atas medium kultur tiruan. Ini telah diperolehi dengan mendidik kulat ini pada mulanya di atas CCM yang kaya dengan pemakanan diikuti dengan medium yang kurang pemakanan, 2% agar air. Kultur-kultur ini dieramkan pada 25 ± 2°C dan diudarakan dengan udara lembap yang tepu.

Morfologi struktur berspora di dalam kultur telah dikaji dibawah mikroskop cahaya dan elektron pengimbasan. Ciri-ciri dan ukuran struktur berspora di dalam kultur adalah amat menyerupai bentuk yang dilihat dalam keadaan semulajadi di ladang di Papua New Guinea dan Malaysia.

Pewarnaan nukleus O. theobromae dengan kaedah Safranin O-KOH menunjukkan bahawa sel-sel tampang, basidiospora dan basidiospora yang bercambah secara berulang di dalam kultur kebanyakannya adalah dwinukleus.

Satu teknik dwikultur kalus koko dengan O. theobromae telah diperkembangkan untuk saringan awal kerintangan terhadap penyakit. Kalus yang mempunyai perhubungan klon yang diketahui adalah genotaip koko yang peka atau rintang, telah dihasilkan dari buku tunas tegak koko yang lembut di atas medium Murashige dan Skoog (1962) yang telah diubahsuai dengan 30 mgl^{-1} IAA dan 5 mgl^{-1} kinetin. Inokulasi kalus dengan O. theobromae telah menunjukkan perbezaan kolonisasi antara genotaip-genotaip koko. Kalus yang dihasilkan dari genotaip yang peka menunjukkan kebolehan kolonisasi yang lebih cepat dan

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Penggunaan teknik dwikultur kalus koko dengan O. theobromae untuk kajian keracunan satu racun kulat sistemik, propiconazole, telah diuji. Perencatan kulat yang beransur didapati berkait dengan peningkatan paras dos racun kulat yang diuji.

CHAPTER 1

INTRODUCTION

The cocoa industry in Malaysia has expanded rapidly within the last two decades. The area under cocoa cultivation has increased to 311000 hectares in 1987 as compared to 2940 hectares in 1965. Production of cocoa beans has also increased from 1000 tonnes to 150000 tonnes over the same period (Ministry of Finance, 1987; Ahmad et al., 1987). Currently Malaysia ranks fourth among cocoa producing countries in the world (Ministry of Finance, 1987). In keeping with the rate of expansion, Malaysia is expected to be the third largest producer by the end of this century. There are a few problems which may act against Malaysia attaining the projected target. Among these there are a few serious pest problems affecting production. Vascular streak dieback (VSD) is presently considered to be the most threatening disease of cocoa in Malaysia and neighbouring cocoa producing countries in Asia (Varghese et al., 1983; Varghese, 1985).

Vascular streak dieback caused by Oncobasidium theobromae Talbot and Keane is a systemic disease. The air borne basidiospores are believed to be the only known infective propagules of the pathogen. The spores penetrate young and unharden flushes of cocoa and grow systemically in the xylem tissues of the leaves, branches and stem (Keane et al., 1972; Prior, 1979). An incubation period of between two to five months is needed for the development of symptoms. Early symptoms are shown by the paling of the leaves

normally in the hardened flush, in advance stage chlorosis and falling of the leaves occur. The fungus also causes brown streaking in the vascular tissues (Keane et al., 1972; Prior, 1979; Zainal Abidin et al., 1981). The fungus sporulates on the abscissed leaf scars during wet weather and the spread of the disease coincides with periods of heavy rainfall (Keane et al., 1972; Keane, 1981; Zainal Abidin, 1982).

The occurrence of VSD in epidemic proportions has been reported recently in many localities in Malaysia, especially in Sabah, and the Philippines (Varghese et al., 1987). Mortality of more than 70% of immature cocoa necessitating repeated planting has not been uncommon in Sabah (Varghese et al., 1987). Apart from losses of immature plantings significant debilitation and dieback of mature plantings can result in serious yield losses. Estimated production loss of 25-40% due to VSD incidence has been reported (Byrne, 1976). Tan (1982) showed that VSD incidence was significantly negatively correlated to yield. In view of the seriousness of the disease, further investigations into the biology and control of the pathogen are necessary.

The biology of O. theobromae has been a subject of much study. However, certain aspects are still unfamiliar and information lacking. These include the loss of survivability of the pathogen and failure to sporulate on artificial culture media. The poor surviving ability of the fungus on culture media has caused prolonged maintenance of O. theobromae in the laboratory almost impossible especially for continuous investigations. In addition, the lack of sporulation on media coupled with the irregular and

sparse availability of basidiospores under natural conditions have hampered research on the host-parasite relationship and the screening of disease resistance materials.

Current methods of controlling VSD are cultural, resistance and chemical control (Mainstone et al., 1983; Prior, 1984a). Sufficient knowledge has been gained for cultural control especially by eradicative pruning. Resistance and chemical control are thought to offer long term and most practical control measures for VSD. Resistant clones are currently planted in Papua New Guinea (Prior, 1978), but these materials are not easily available in other countries. This has necessitated the need to carry out resistance screening of locally available cocoa clones and progenies. Presently, no rapid and reliable technique is available for mass screening of resistance in which its development is desirable. Investigations into the possible resistance mechanisms against VSD will provide better understanding of the host- parasite interaction, as well as identify features which could be associated with resistance. Chemical control has not found much favour as previously the fungicides tested had not been effective (Prior, 1980). However, recently systemic fungicides tested belonging to the triazole group had shown promising indications especially in reducing significant disease incidence in cocoa nurseries and field plantings (Mainstone et al., 1983; Chung, 1983; Varghese et al., 1985; Gurmit, 1986; Prior, 1986; Sidhu, 1987). Further screening work need to be done to select more suitable chemicals for control of VSD.